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Maintenance of sterility in SMS packaging in dental environments: concern with biosafety

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ABSTRACT. Healthcare services must be guided by biosafety practices and microbial control. This control is highly influenced by humidity, which directly impacts the maintenance of sterility of the materials used in the appointments. High concentration of moisture, in the form of aerosol, splashes and spills, is caused during dental care. During the COVID-19 times the contamination by aerosol and droplets worries greatly. Considering that it could cause harm to the sterility of an autoclaved material, especially in dental environments, the objective was to evaluate the behavior of SMS sterilization packages (Spunbonded / Meltblown / Spunbonded) against microbial penetration in an aqueous vehicle. SMS of three brands were challenged, equally divided into two groups: virgin and processed (subjected to a single autoclaving cycle). Each specimen was aseptically deposited on Macconkey agar. Subsequently, 5 µL of *Escherichia coli* ATCC 25922 saline solution [10^8 CFU mL^{-1}] was deposited in center of the SMS specimen and the dish incubated at 36°C / 48h. Reading was performed by the presence or absence of bacterial growth typical of the species under the SMS, observed on the back of Petri dish. The lowest penetration rate observed was 60% for one of the brands in the virgin condition, and 75% for two brands in the processed condition. Statistical analysis showed an association between bacterial penetration and the evaluated group, this association being valid only in the virgin condition. The different SMS behave similarly in terms of resistance to bacterial penetration after being processed. The data show that moisture can assist in bacterial transport through sterilized SMS. Therefore, SMS packages are not able to prevent bacterial penetration, and possibly other microorganisms, when in aqueous vehicles, offering a potential risk of breaking the aseptic chain. Thus, care must be taken in routines for handling and storage sterile packaging.

Keywords: infection control; cross infection; humidity; aerosol; dental droplets; microbial control.

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Introduction

Providing safe care to patients is a common pursuit of health services such as doctor's offices, clinics, hospitals and academic care centers. To achieve this goal, the sterilization of materials is a primary resource in such establishments, with the respective professionals responsible for processing the materials that must be carefully performed so that sterility is guaranteed (Serratine, Gonçalves, & Luçolli, 2009). However, the sterility of a product does not depend solely on the sterilization process, but covers the entire processing chain up to the storage of the sterilized material. Therefore, the consistency of sterilization requires a comprehensive program of aseptic practices, among which, avoiding humidity is a fact mentioned in the literature, because it can bring with it microorganisms from the air and surfaces (Rutala, Weber, & Healthcare Infection Control Practices Advisory Committee, 2008; Rutala & Weber, 2019).

Sterilized products must be stored in a clean and dry place, under the protection of direct sunlight, and subjected to minimal manipulation. Furthermore, the person responsible for the Materials and Sterilization Center must establish rules for the control of events that may compromise integrity and sealing the packaging of health products. In addition, when transporting processed health products, the procedure must be carried out in closed containers and in conditions that guarantee the maintenance of the identification and integrity of the sterilization packaging (Brasil, 2012).

According to the relevant literature, an ideal packaging material for sterilization should allow the sterilization process, maintain their sterility until they are opened, guarantee the integrity of the material during transport, resist moisture preventing its penetration into the package , be flexible facilitating its handling and have a low cost (Costa, 2004; Souza, Sória, Araújo, Silva, & Andrade, 2010; Oliveira, Costa, Zocratto, & Branco, 2011).

The SMS (Spunbonded / Meltblown / Spunbonded) is a material that meets the listed requirements and is routinely used in sterilization services, not being considered a fabric, as it does not present an interweaving of a longitudinal warp accompanied by a perpendicular filament weave along material axis. Therefore, SMS is classified as a non-woven with a flat, flexible and porous structure, consisting of a veil or blanket with filaments or unorganized fibers (Associação Brasileira de Normas Técnicas [ABNT], 2017). Such a product is generated through patented processes called Spunbonded / Meltblown / Spunbonded. The final structure of the SMS nonwoven features two layers of high-strength polypropylene from Spunbonded processing interspersed by a layer with high filtration power of the Meltblown process (Epps & Leonas, 2000).

In dental offices, the use of high-speed turbines, ultrasonic scrapers and air/water syringes causes the formation of aerosol (Discacciati, Sander, Castilho, & Resende, 1998). Aerosol means a solution (often liquid) suspended in the air. These particles can remain floating for a short or long period of time, depending on their size, which can vary between 0.001 and 10,000 µm. Aerosol particles with diameters larger than 100 µm are called spatters and, due to the gravitational force, settle more quickly than smaller particles (Samaranayake, Scheutz, & Cottone, 1995). The related literature accepts that small particles of aerodynamic diameter < 5 – 10 µm, which follow airflow lines, are potentially capable of short- and long-range transmission (Tellier, Li, Cowling, & Tang, 2019). Thus, pathogenic microorganisms, which may be present in patients' blood and saliva, can be transported by aerosol and spread an infection (Bentley, Burkhart, & Crawford, 1994, Samaranayake et al., 1995, Discacciati et al., 1998).

With the advent of COVID-19, the presence of moisture, especially in the form of aerosols and splashes, proved to be critical for infection control. In addition, the presence of the virus in the oral cavity characterizes a great risk of spreading contamination in dental offices, since droplets generated by infected people are the main form of transmission of the virus (Peng et al., 2020; Xu et al., 2020).

The Sars-Cov-2 pandemic made the role of aerosol and spills as diffusion spreaders much more evident (Anderson, Turnham, Griffin, & Clarke, 2020; Izzetti, Nisi, Gabriele, & Graziani, 2020; Meng, Hua, & Bian, 2020, Peng et al, 2020). In addition, the concern with surfaces gained more prominence, due to the epidemiology of infection transmission, making it possible to detect the new human coronavirus on inanimate surfaces for up to nine days (Kampf, Todt, Pfaender, & Steinmann, 2020).

In view of the above, and considering that conceptually, if sterilized SMS packaging comes into contact with moisture, there could be a compromise in the sterility of an autoclaved material, especially in dental environments in COVID-19 times, the objective of the present study was to evaluate the behavior of SMS against microbial penetration in an aqueous vehicle. The null hypothesis is that there is no association between bacterial penetration in an aqueous vehicle and the condition of SMS.

Material and methods

Sample characterization

Three different brands of SMS for dental-medical-hospital use, all of the same weight (40gr m^{-2}), normally marketed, were used. Eighty specimens, 8 cm in diameter each, were obtained from the different SMS brands (experimental n = 240). The specimens of each SMS brand were then divided into two groups (n = 40 for each brand / total n = 120 per group): virgin SMS (without sterilization) and processed SMS (with sterilization), as shown in figure 1. The sterilization of the processed group was obtained by submitting surgical boxes packed with the study SMS to the autoclaving process using an autoclave SERCON HS 10360 at 134°C, for 10 min. at 2.1 ATM, with an additional drying cycle.

Test microorganism

As a microorganism model for the study, the strain of *Escherichia coli* ATCC 25922 was used. Thus, fresh culture of the bacteria was obtained by growing overnight at 36°C in Macconkey Agar. To obtain the inoculum, 3-5 colonies were then suspended in sterile saline (NaCl 0.8%), adjusting the optical density to approximately 10^8 CFU mL^{-1} with the aid of the 0.5 standard of the McFarland scale.

Verification test for bacterial penetration via SMS by aqueous vehicle

Each specimen was deposited aseptically on the surface of the solid Macconkey culture medium. For the group of processed SMS, the inner side of the packaging of the surgical boxes, therefore sterile, was placed in contact with the culture medium. In the virgin SMS group, these specimens were first decontaminated on the one hand by means of UV radiation. This same side was then placed in contact with the culture medium. Then, in the center of the 240 specimens, an inoculum of 5 µL of the bacterial culture previously adjusted (10^8 CFU mL^{-1}) was deposited. As a control, the specimen itself was used in the regions around the inoculum point. All test plates (n = 240), 80 from each SMS brand, were then incubated at 36°C for 48 hours. An assay scheme is shown in Figure 1. The possible bacterial passage was read by checking the presence or absence of bacterial growth in the culture medium, in the center of the Petri dish, below the specimen, detected by mass cell with pink color, characteristic of *E. coli* culture in Macconkey medium, observed by the reverse of the Petri dish.

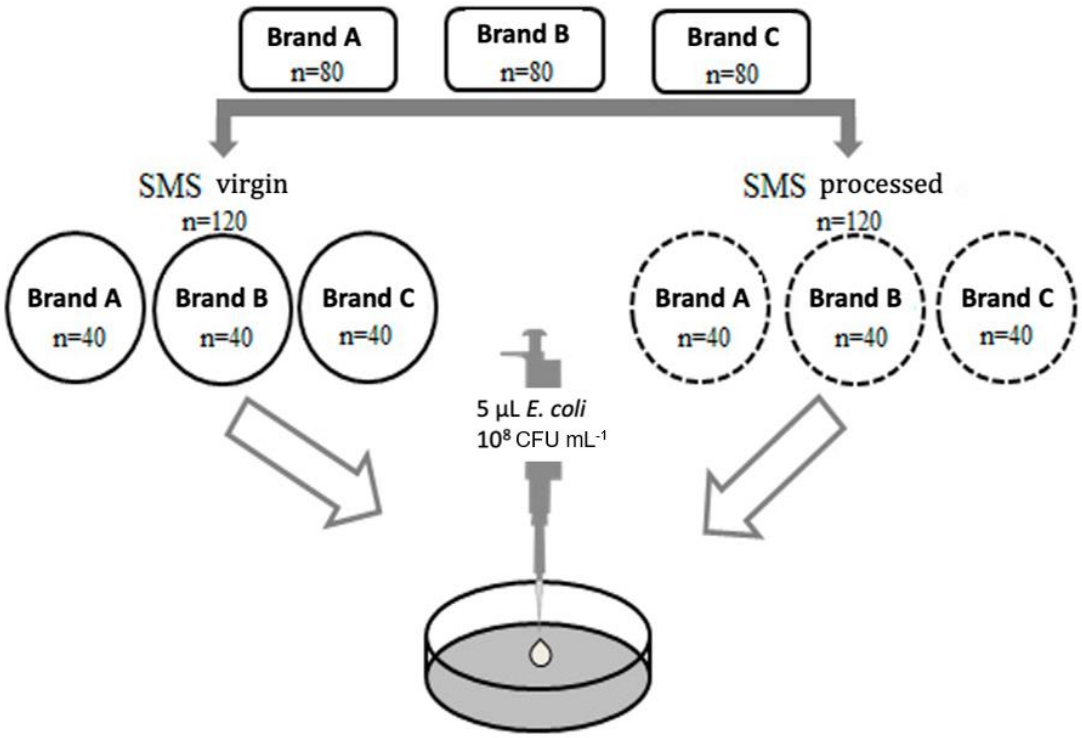

Figure 1. Schematic flow of the organization of the SMS groups for the study, considering the three commercial brands used (A, B and C), and the two tested SMS conditions which are: 1) virgin or 2) previously autoclaved (processed).

Data analysis

The analyzes were performed using the statistical software SPSS version 21.0 (SPSS, Chicago, IL, USA). Descriptive frequency analyzes were performed and the chi-square test of independence was used to assess the association between the variables brand and condition (virgin and processed) of the SMS with the outcome (penetration or not of the bacterium). The level of significance used was 0.05 ($p < 0.05$).

Results and discussion

Figure 2 shows the relative frequencies (%) obtained by observing bacterial growth in the culture medium plates, in the specimens, separated by brand and condition (virgin and processed) of the SMS.

The statistical analysis showed that there is no association between the condition (virgin and processed) of the SMS and the outcome in bacterial penetration ($p = 0.337$) when considering all the results regardless of the brand. However, when considering the six groups, separated by the brands and the condition of the SMS, there is an association ($p = 0.036$) between bacterial penetration and the evaluated group. In order to detect where this association is, an analysis was performed comparing the three brands first in the condition of use ($p = 0.929$) and then in the virgin condition ($p = 0.001$). Thus, it was found that the association between bacterial penetration and the SMS brand is only valid before autoclaving (virgin condition), and that after sterilization by this process, the SMS behave similarly in terms of resistance to bacterial penetration.

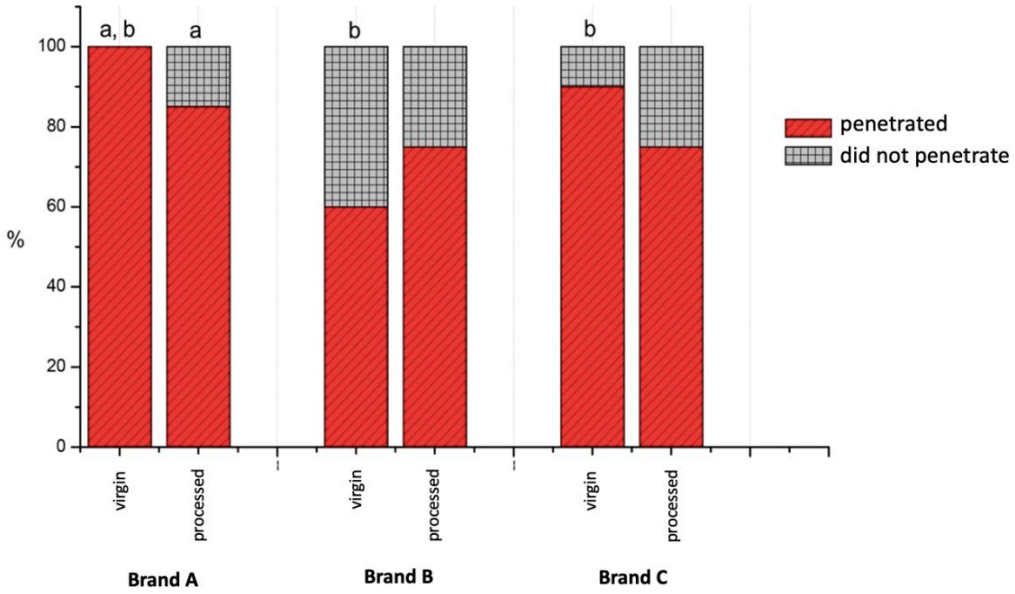

Figure 2. Distribution of the frequencies (%) of bacterial penetration in the different SMS brands for both the virgin and the processed condition. The letters indicate a statistical difference in bacterial penetration between the SMS condition for the same brand (a) and between the brands for the same condition (b).

It was also evaluated whether, for the same brand, there is an association of penetration with the SMS condition, obtaining the following p values: brand A = 0.035, brand B = 0.311 and brand C = 0.184. Thus, there is an association of bacterial penetration with the condition of SMS only for the A brand.

The concern with the safety of the patient and dental care workers must be considered as a primary factor for the actions performed in the clinical routine. Reducing risks requires the incorporation of good practices in health services, actions that guarantee the effectiveness of the care provided and the safety of those involved (Oliveira et al., 2014, Brasil, 2020).

Cross infection occurs through person-to-person contact, through air and its constituents or through contaminated objects (Fantinato, 1994; Samaranayake et al., 1995). Splashes and aerosols are responsible for generating cross-infection, as they carry pathogenic microorganisms (Wei & Li, 2016). A splash can travel up to two meters and thus reach dental work surfaces (Discacciati et al., 1998), where SMS packages of sterile material may be present. Considering that the ability of Sars-CoV-2 to remain viable and infectious in aerosols for hours and surfaces for days (Doremalen et al., 2020; Kampf et al., 2020) in times of the COVID-19 pandemic, contamination by aerosol or splashes is of concern.

Non-woven fabrics for dental-medical-hospital use must have their production inspected, with manufacturers responsible for attesting their characteristics. In Brazil, medical, hospital and dental materials must be subject to sanitary control and inspection by Anvisa, in accordance with Law No. 9,782, and the health surveillance regime also extends to the physical facilities, equipment, technologies, environments and procedures involved in all the phases of the production processes of goods and products (Brasil, 1999), in the form of health licensing (Brasil, 2017). Specifically the packaging for sterilization, defined by standard NBR 14858 as "[...] single-use article, made of non-woven fabric, used to allow sterilization, maintain the sterility of the content until the package is opened and enable the delivery of the content without contaminating it [...]" (Associação Brasileira de Normas Técnicas [ABNT], 2010, p. 2), must be regularized by Anvisa, for specific use in sterilization (Brasil, 2012).

Considering the microbial penetration and the presence of humidity, although a test is foreseen to determine the resistance of the penetration under conditions defined by NBR 15622 (ABNT, 2008), it is not clear whether these tests should always be performed for all batches of SMS destined for sterilization packaging. The results of this work are alarming, considering the frequency of bacterial penetration regardless of whether the SMS has already undergone an autoclaving process. It should be noted that the test is not intended to attest to the quality of the SMS assessed here and, therefore, the microbial challenge to penetration was much lower than expected in the standard. The objective was to simulate the contact of a small fraction of contaminated liquid, represented here by 5 µL of bacterial suspension, to already sterilized packages. While in the standard the procedure also uses a bacterium (*Staphylococcus aureus* ATCC 29213) and the specimen is, as in the test performed, placed on the solid culture medium, a force of 3N is still applied for

15 minutes (on a permeable film carrier of the bacteria and juxtaposed to the specimen) and, therefore, the challenge is even greater. Thus, a much lower penetration result was expected than was observed.

In this study, the *E. coli* species was chosen instead of *S. aureus* because its growth on Macconkey agar is easily detected, in addition to the selectivity of this culture medium that inhibits Gram-positive species, which would be primarily the contaminants of the species process during handling and by the presence of the operators' skin resident microbiota. It should also be noted that the reduced volume of inoculum (5 µL) represents only 10% of the volume of a standard drop (50 µL), and although still much higher than the aerosol, it is difficult to visualize and control, especially during routine clinical procedure.

At dental schools where the processing of materials is carried out in a decentralized manner, students perform the steps of purge, preparation and storage, the risk of failure in the process increases considerably when compared to the risk of processing at Material and Sterilization Centers - responsible through all stages (Reis, Ramos, Zocratto, & Branco, 2012). Decontamination practices and the control of surface humidity certainly present an even greater challenge with these academic centers. Tipple, Silva, Paiva, Pereira, and Moriya (2004), affirm that it is a routine practice for students to store their sterile materials in bins, along with other non-sterile objects, and to not perform the proper cleaning of the environment. They observed dentistry undergraduates who said they used the cabinets to deposit different types of articles for clinical use such as PPE, molding materials and even materials used to wash infected articles. This observation must be taken into account, especially in the return of the universities in the post-pandemic. It is important to note that the current resolution determines the storage of processed materials in a clean and dry place, avoiding direct sunlight and being subjected to the minimum possible manipulation (Brasil, 2012). Despite being considered not very complex, failures in this stage can result in the compromise of the entire aseptic chain (Prado & Santos, 2002).

The data show that although the brands show different performances in terms of bacterial penetration, their behavior, in the post autoclaving condition, in terms of resistance to penetration is similar between them ($p = 0.929$). This result is in accordance with the recommendation in standard NBR 15622 (ABNT, 2008) in which the performance of the predicted tests must be after the sterilization process recommended by the manufacturer. Even though, the quality of each SMS must always be evaluated, as the A brand was worse in both the virgin and the processed condition, despite its performance having significantly improved after autoclaving ($p = 0.035$). Regardless of these comparisons between brands, it should be emphasized that a high penetration rate was observed, even after sterilization, where in the best performance (brands B and C) penetration was observed in 75% of the specimens (Figure 2). Thus, the results observed in the present work underscore the need to redouble attention to the possibility of moisture on the surfaces on which sterilized SMS packages are deposited and not just to trust that SMS will prevent microbial passage. This fact is in agreement with the statement that the support surfaces used by the professional must be safely disinfected, dry and free of splashes from previous visits, to avoid cross contamination (Jorge, 2002). There is also the need for prior disinfection of the hands, followed by drying and contact with wet hands is totally inadvisable, as contamination can be released into the environment by aerosols and particles and transferred from surfaces to the hands of health professionals (Otter et. al., 2016).

According to the data presented here, moisture can assist in carrying contamination to internal portions of sterilization packages, compromising it, as well as facilitating the carrying of contamination to other surfaces and environments. Although the *E. coli* bacterium was used in the tests, with a size of 1.1–1.5 × 2.0–6.0 µm (Scheutz & Strockbine, 2015), it should be noted that viruses are much smaller than bacteria, and that, therefore, their penetration would be facilitated. Considering the ability of the Sars-CoV-2 virus to survive for so long in the environments, in addition to the extremely high degree of spread of the infection (Cui, Li, & Shi, 2019; Bai, Nie, & Wen, 2020), the importance is emphasized surface cleaning (Doremalen et al., 2020) and especially the care with sterile packaging. In order to control contamination in sterile packages, health services must have internal protocols containing guidelines to be implemented in all stages of surface cleaning and disinfection and ensure the periodic training of the teams involved, whether they are outsourced or themselves (World Health Organization [WHO], 2020; Brasil, 2020, Conselho Federal de Odontologia [CFO], 2020). The literature also documents that airborne contamination can be minimized with ease and low cost, placing various infection control steps in the routine precautions used during all dental procedures, including the use of high-powered suction pumps (Harrel & Molinari, 2004).

Conclusion

The SMS packages studied were not able to prevent bacterial penetration when in aqueous vehicles. Therefore, it is necessary to have an effective control protocol, followed by drying the surfaces that may come into contact with sterile packages, including contact during transportation, handling and storage.

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